EXHIBIT 8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Cell Populations which Co-Express CD49c and CD90	**Confirmation No.:** 4326
Application No.: 09/960,244	**Art Unit:** 1651
Filed: Sept. 21, 2001	**Examiner:** Leon B. Lankford, Jr.
Assignee: Neuronyx Inc.	**Atty. Docket:** 2560.0020000

Declaration of Gene Kopen Under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, Gene C. Kopen, declare and state as follows:

A. INTRODUCTION AND SUMMARY

1. I received a B.A. degree in Social & Behavioral Sciences from the University of South Florida (Tampa, FL) and a Ph.D. in Molecular Pathobiology from Drexel College of Medicine (Philadelphia, PA). My training continued as a post-doctoral fellow at Hahnemann University (Philadelphia, PA). A copy of my curriculum vitae is attached as **Exhibit A.**

2. I am a co-inventor of the presently claimed invention (U.S. Patent Application No. 09/960,244). I am also currently employed at Neuronyx Incorporated (assignee of the presently claimed invention), where I hold the position of Vice President, Technology Development and Intellectual Property Management. My responsibilities in this capacity include: directing and supervising manufacturing, process development, and quality systems;

managing and directing all patent matters; working with executive management to develop, implement, and oversee execution of strategic plans; participation in developing strategic research and development objectives; developing and implementing new technology initiatives; supporting business development efforts; and, establishing and managing a network of technical experts, industry thought leaders, and academic collaborators to support clinical development of product.

3. I am familiar with the above-identified patent application and pending claims as well as the October 5, 2007 Office Action (Paper No. 20070930) issued in relation to this application.

4. I have been told by attorneys for Neuronyx that the specification of a patent application describes the claimed invention while the claims establish the scope of the invention. I understand that presently pending claims 14, 21 and 25-26 are directed to isolated cell populations derived from human bone marrow wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90 and wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings.

5. I have been asked to give my opinion on whether the isolated cell populations described and claimed in the present patent application are the same, or are essentially the same, as cell populations described in a patent issued to Haynesworth *et al.*, U.S. Patent 5,733,542 (1998) (hereinafter "Haynesworth"), a publication by Pittenger *et al.* (*Science 284*: 143-147 (1999)) (hereinafter "Pittenger"), a publication by Woodbury *et al.* (*Journal of Neuroscience Research 61*: 364-370 (2000)) (hereinafter "Woodbury"), a publication by Lee *et al.* (*Hepatology 40*: 1275-1284 (2004)) (hereinafter "Lee"), or a publication by Jiang *et al.* (*Nature 418*: 41-49 (2002) (hereinafter "Jiang").

6. It is my opinion that the cells described and claimed in the present patent application are not the same, nor are they essentially the same, as the cell populations described in the publications referenced in (5) above. In addition to the description provided in the present patent application, this opinion is also based on additional experimental

evidence described herein, as well as based on data described in my previous Declaration (submitted May 18, 2007 in this same application; hereinafter the "May 2007 Declaration"), and as documented knowledge commonly known to those of ordinary skill in the art.

B. THE CELLS DESCRIBED AND CLAIMED IN THE PRESENT PATENT APPLICATION ARE NOT THE SAME, NOR ARE THEY ESSENTIALLY THE SAME, AS THE CELL POPULATIONS DESCRIBED IN HAYNESWORTH

1. Haynesworth's "MSC" cell population expresses different cell surface markers compared to the cell populations of the present invention.

I have been advised by attorneys for Neuronyx that the Haynesworth '542 patent is a continuation-in-part of a patent issued to Caplan *et al.* (U.S. Patent 5,486,359; Appln. Ser. No. 193,262; hereinafter "Caplan '359"). Moreover, at column 1, lines 24-31, Haynesworth states that "the inventors of the present invention have discovered a process for isolating, purifying, and greatly replicating the mesenchymal stem cells in culture, *i.e. in vitro.* This discovery is the subject of co-pending U.S. patent application Ser. No. 193,262, filed Feb. 8, 1994 [*i.e.*, Caplan '359]." Hence, it is apparent that the description in Haynesworth relies on the description in Caplan '359 for the methods used to isolate the cells described in Haynesworth.

Likewise, I have been advised by attorneys for Neuronyx that another patent issued to Caplan *et al.* (U.S. Patent 5,811,094; hereinafter "Caplan '094") is also a continuation-in-part of Caplan '359. Indeed, it is clear that Caplan '094 teaches isolation of the same cell population as Caplan '359 because Example 1 ("The Isolation, Purification and Culture-Expansion of Human Mesenchymal Stem Cells") in both of these patents teach the same methods of isolating human "MSCs."

Hence, the "MSCs" of Haynesworth are the same as those described in Caplan '359 and Caplan '094. Accordingly, the cells in each of these patents will have the same characteristics even if such characteristics are mentioned in one patent but not the other. In this regard, in addition to characteristics described in Haynesworth and Caplan '359, Caplan

'094 further teaches additional cell surface marker profiles of the isolated "MSC" cell population. *See*, Caplan '094, Example 4, columns 37 to 39. In particular, Table 5 (column 39) in Caplan '094 teaches that the isolated "MSC" cell population is *CD44 negative.*

In contrast, my May 2007 Declaration demonstrated that the cell populations of the present invention are *CD44 positive.* Hence, the CD44 negative "MSC" cell population isolated by Haynesworth is a different cell population from the CD44 positive cell populations of the present invention.

2. Haynesworth's "MSC" cell populations are obtained from a low density gradient fraction whereas cell populations of the present invention are obtained from a high density gradient fraction.

Haynesworth teaches that their adherent "MSC" cells are localized within a population of "*low density* cells" corresponding to the *platelet fraction* of bone marrow-derived cells. In contrast, the cell populations of the present invention are isolated from a *high density mononuclear fraction* (discussed further below). In particular, the bone marrow aspirate/70% Percoll gradient fractionation procedure used by Haynesworth is described as follows:

> Aspirate marrow (5-10 ml) was transferred to sterile tubes to which 20 ml complete medium was added. The tubes were spun at 1000xRPM for 5 minutes to pellet the cells. The supernatant and fat layer were removed and the cell pellets (2.5-5.0 ml) were loaded onto 70% Percoll (Sigma, St. Louis, Mo.) gradients and spun at 460x g for 15 minutes. The gradients were separated into three fractions with a pipet: *top 25% of the gradient (low density cells-platelet fraction), pooled density=1.03 g/ml; middle 50% of the gradient (high density cells-mononucleated cells), pooled density=1.10 g/ml*; and, bottom 25% of the gradient (red blood cells), pooled density=1.14 g/ml. In preliminary experiments each of these three pools were plated separately in complete medium in 100 mm dishes.[1] *Adherent cells were observed to be localized to the low density cells.* To produce adherent cell cultures for all subsequent experiments, *only the low density cells were plated.*

[1] Caplan '359 teaches that their "MSCs" are found in the low density fraction of cells at a concentration of about 50-500 "MSCs" per 30-50 x 10^6 nucleated cells (or a concentraton of about 0.001% to 0.00016 % MSCs). *See*, Caplan '359, col. 10, lines 5-9. In contrast, the isolated cells of the present invention are found in high

See, Caplan '359, column 17, lines 7-24 (emphasis added).

In addition to isolation of their cell populations from low density gradient fractions, the fractionation media used by Haynesworth versus that used in the present specification are substantially different, further indicating that Haynesworth's cell populations are different from those of the present invention. In particular, Haynesworth uses *70% Percoll* fractionation media, whereas the media used to isolate cells of the present invention is *Histopaque 1.119®*. This is significant because Histopaque 1.119® is typically used by itself to isolate *high density* cells, such as nucleated red blood cells, granulocytes, and polymorphonuclear cells (PMNs), from bone marrow or peripheral blood. In contrast, the separation of a *low density* cell fraction at 1.03g/ml (where Haynesworth's "MSCs" are reportedly localized) cannot be achieved using Histopaque 1.119® alone. Instead, to isolate the lower density cells of Haynesworth, Histopaque 1.119® would have to be combined with a lower density reagent, such as Histopaque 1.077® (see **Exhibit B**, 3rd paragraph, "Intended Use"). Thus, use of Histopaque 1.119® alone, as described in the present specification could not be used to fractionate the low density "MSCs" of Haynesworth because, following gradient centrifugation, Haynesworth's "MSCs" would be found in an unfractionated plasma layer of cells on top of the high-density Histopaque 1.119® media. *See*, **Exhibit B**; figure at step 6.

In contrast to Haynesworth, the cell populations of the present application are isolated from high density mononucleated cell fractions obtained through use of relatively high density fractionation media. In particular, the 09/960,244 specification teaches:

> Bone marrow cells were aspirated from the iliac crest of healthy adult human volunteers. The bone marrow aspirate was diluted with calcium and magnesium free phosphate buffered saline (PBS) to achieve a mononuclear cell concentration of $7x10^6$ cells/mL and overlaid onto an equal volume of *Histopaque®1.119* (Sigma, St. Louis, Mo.) and centrifuged (30 min at 700xg). The resulting *mononuclear cell fraction* was transferred to a clean centrifuge tube containing PBS and centrifuged (10 minutes at 500xg). The cell pellet was re-suspended in PBS and centrifuged (10 minutes at 500xg). The

density mononuclear cell gradient fractions at concentrations of ~1 CD49c/CD90 positive cell per 200 total cells or ~0.5%. *See*, **Exhibit E.**

> supernatant was aspirated from the cell pellet and the cells re-suspended in complete media.

See, Specification, page 27, lines 9-19 (emphasis added).

Therefore, since the cell populations of the present application are isolated from high density mononuclear cell fractions (in contrast to the low-density platelet fractions where Haynesworth's cells are found), the isolated population of cells described by Haynesworth and those of the present application are different starting populations of cells, even before any subsequent seeding/plating/culturing procedures are carried out.

3. Haynesworth teaches that isolation of their cell population requires specific media which is "critical" for isolation of their "MSCs".

Isolation of Haynesworth's "MSC" cell population is taught to require specific media which provides "*the critical step*" for isolation "*of only the mesenchymal stem cells.*" For example, Caplan '359 (upon which Haynesworth relies in the process for isolating and purifying their "MSC" cells (*see* Haynesworth, col. 1, lines 27-31)) teaches:

> The method of their isolation comprises the steps of providing a tissue specimen containing mesenchymal stem cells, adding cells from the tissue specimen to *a medium which contains factors that* stimulate mesenchymal stem cell growth without differentiation and *allows, when cultured, for the selective adherence of only the mesenchymal stem cells* to a substrate surface, culturing the specimen-medium mixture, and removing the non-adherent matter from the substrate surface.
>
> In another aspect, the present invention relates to a medium for isolating human mesenchymal stem cells from a tissue specimen, wherein the *medium is comprised of factors which* stimulate mesenchymal stem cell growth without differentiation *and allows, when cultured, for the selective adherence of only the mesenchymal stem cells* to a substrate surface.

See, Caplan '359, column 2, lines 22-36 (emphasis added).

> These cells are referred to by the inventors as human "mesenchymal stem cells" or "MSCs." In this regard, it has been found that although these progenitor mesenchymal stem cells are normally present in bone marrow, for example, in very minute amounts and that these amounts greatly decrease with age (i.e. from about 1/10,000 cells in a relatively young patient to as few as 1/2,000,000 in an elderly patient), *human mesenchymal stem cells can be*

> *isolated from tissue and purified when cultured in a **specific** medium by their selective attachment, termed "adherence," to substrates.*

See, Caplan '359, column 4, lines 24-34 (emphasis added).

> Although the harvested marrow was prepared for cell culture separation by a number of different mechanical isolation processes depending upon the source of the harvested marrow (i.e. the presence of bone chips, peripheral blood, etc.), ***the critical step*** involved in the isolation processes was the use of a ***specially prepared medium*** that contained agents which allowed for not only mesenchymal stem cell growth without differentiation, but also for the direct adherence ***of only the mesenchymal stem cells*** to the plastic or glass surface area of the culture dish. By producing a medium which allowed for the selective attachment of the desired mesenchymal stem cells which were present in the marrow samples in very minute amounts, it was possible to separate the mesenchymal stem cells from the other cells (i.e. red and white blood cells, other differentiated mesenchymal cells, etc.) present in the bone marrow.

See, Caplan '359, column 6, lines 22-37 (emphasis added).

Caplan '359 specifically describes three types of media which support "the critical step" of selective adherence of "MSCs" to the cell culture substrate. These media are identified (along with their constituent components) as: "Dulbecco's Modified Eagle's Medium", "BGJb Medium (Fitton-Jackson Modification)", and "F-12 Nutrient Mixture (Ham)". *See*, Caplan '359, column 6, line 45 through column 9, line 35.

In contrast, the media described for use in isolating and culturing cell populations of the present invention is not the same as any of the three media described for isolation of Haynesworth "MSCs." Attached **Exhibit C** shows a comparison of components in the media used to isolate and culture cells of the present invention (*i.e.*, "Minimal Essential Medium-alpha (Gibco BRL, Rockville, MD) supplemented with 4mM glutamine" (minus FBS)) compared to the components in the three types of media described in Caplan '359. *Compare e.g.*, 09/960,244 specification at page 25, lines 17-21 *with* Caplan '359, column 6, line 45 through column 9, line 35.

As demonstrated by the attached **Exhibit C**, each of the three media described in Caplan '359 either *has components not found* in the media used to isolate and culture cells of the present invention, *is missing components that are found* in media of the present specification, and/or *has different concentrations of components* compared to media of the present specification. In other words, since the step that is described as "critical" for isolating Haynesworth's cell populations, is not the same as that used to isolate cell populations of the present invention, it is improbable (on this basis alone) that the cell populations of Haynesworth are the same as those of the present invention.

4. Haynesworth's cells have longer population doubling times and diminished self-renewal capacity than the cell populations of the present invention.

Characteristics of the "MSCs" described in Haynesworth are also further descibed in another paper co-authored by Haynesworth, "Growth Kinetics, Self-Renewal, and the Osteogenic Potential of Purified Human Mesenchymal Stem Cells During Extensive Subcultivation and Following Cryopreservation," Bruder, Jaiswal & Haynesworth, *Jour. Cell. Biochem. 64*:278-294 (1997) (hereinafter "Bruder"). This is evident, *inter alia*, because the methods used in obtaining the "MSCs" in Bruder are the same as those described in Haynesworth '542. *Compare*, Haynesworth '542, Example 1, col. 3, line 57 to col. 4, line 51 *and* Caplan '359, Example 1, col. 17, lines 7-45 *with* Bruder, page 279, right col., last paragraph to page 280, left column, first paragraph.

Among such characteristics, the "MSCs" of Haynesworth are descibed as acquiring increasingly longer population doubling times with each passage in culture and also of having an average self-renewal capacity of only 38 +/- 4 population doublings (with the cells, thereafter, "degenerating"). *See, for example*, Bruder at:

Abstract:

> The average number of population doublings for marrow-derived adult human MSCs was determined to be 38 +/- 4, at which time the cells finally became very broad and flattened before degenerating... The use of population doubling potential as a measure of biological age suggests that MSCs are intermediately between embryonic and adult tissues.

Page 284, left column, second paragraph:

With increasing passage number, the hMSC growth rates were slower and the number of cells generated by the end of 9 days in culture was reduced. Figure 4 illustrates that by passge 4, a decline in the number of cells generated during the 9 day culture period was observable... At passage 7 and beyond, the growth of cells had waned in comparison to passage 1 cultures at nearly every time point beyond day 5 ($P<0.05$). This slowing of cell proliferation as a function of increasing passage number was independent of the feeding schedule used to maintain the cells.

Page 284, right column, last paragraph to page 285, left column, first paragraph:

The mean cumulative population doublings for the four donor hMSC preparations was 38 +/- 4 (Fig. 5). On average, 11.2 population doublings took place in primary culture, accounting for 29.4% of the mean cumulative number of population doublings... After the tenth passage, the number of population doublings declined as senescence was reached in the different donor preparations. By passage 12, hMSCs in two of the four donor preparations had stopped dividing, while cells in the other two cultures continued to divide very slowly and were passaged prior to achieving 90% confluence to determine if replating would stimulate further cell division. As observed in Figure 5, few additional population doublings were generated by cells subcultured from passages 12 through 15.

Page 285, left column, last paragraph:

As passage number increased, the time between initial plating and subsequent subculturing increased from 5 days to approximately 10 days by the tenth passage.

In sum, Bruder describes Haynesworth's "MSCs" as exhibiting reduced capacity for proliferation and increasingly longer population doubling times with each passage in culture.

The doubling times of Haynesworth's cell populations not only become progressively longer with each passage in culture, but the doubling times are also *initially* longer than 30 hours/population doubling. As demonstrated in the table and calculation shown below, based on the doubling times described for, and depicted in, Figure 4 in Bruder (page 284), Haynesworth's human "MSCs" have average population doubling times ranging, at best, from approximately 54 to more than 100 hours/population doubling.

A	B	C	D	E	F
Passage Number	**Number of cells seeded at start of indicated passage.**	**Number of cells ("yield") at end of indicated passage:**	**Days in culture during indicated passage.**	**Number of population doublings during indicated passage.**	**Population doubling rate during indicated passage** (number of days in culture x 24 hrs. / number of pop. doublings).
P1	19,240 cells	$2.7x10^5$	9	~4	**~54** hours/pop. doubling
P4	19,240 cells	$1.1x10^5$	9	~3	**~72** hours/pop. doubling
P7	19,240 cells	$0.88x10^5$	9	~2.5	**~86** hours/pop. doubling
P10	19,240 cells	$0.68x10^5$	9	~2	**~108** hours/pop. doubling

Columns A/B: **Calculation of number of cells seeded at start of indicated passage.**

1. "*At each passage*, cells were seeded at *$2x10^3$ cells per cm^2 in 35mm plates* and fed on a twice weekly (a) or daily (b) schedule." *See*, Bruder, page 284, Fig. 4, legend (emphasis added).
2. One 35mm plate has an area of $\pi R^2 = 3.14 \cdot (3.5cm/2)^2 = 9.62\ cm^2$.
   $2x10^3$ cells per 9.62 cm^2 = 2000 cells • 9.62 cm^2 = **19,240 cells seeded** (or 0.1924×10^5 cells).

Columns C/D: **Calculation of number of cells at end of indicated passage.**

"For example, 9 days after replating cells derived from **passages 1, 4, 7, and 10**, the mean yields for twice weekly fed cultures were **$2.7x10^5$, $1.1x10^5$, $0.88x10^5$, and $0.68x10^5$ cells, respectively."** *See* Bruder, p. 284, left col., 2nd para (emphasis added).

Column E: **Calculation of number of population doublings during indicated passage.**

1 population doubling starting with 19,240 cells:
0.1924×10^5 cells **x 2** = 0.3848×10^5 cells = **1** population doubling

2 population doublings starting with 19,240 cells:
0.1924×10^5 cells x 2 = 0.3848×10^5 cells = 1 population doubling
0.3848×10^5 cells x 2 = **0.7696×10^5 cells = 2** population doublings

Bruder describes mean yield of cells at end of **P10** as = $0.68x10^5$ cells
Given that 0.7696×10^5 cells = 2 population doublings, then $0.68x10^5$ cells = **~2 pop. doublings.**

Bruder describes mean yield of cells at end of **P7** as = $0.88x10^5$ cells
Given that 0.7696×10^5 cells = 2 population doublings, then $0.88x10^5$ cells = **~2.5 pop. doublings.**

3 population doublings starting with 19,240 cells:
0.1924×10^5 cells x 2 = 0.3848×10^5 cells = 1 population doubling
0.3848×10^5 cells x 2 = 0.7696×10^5 cells = 2 population doublings
0.7696×10^5 cells x 2 = **1.5392×10^5 cells = 3 population doublings**

Bruder describes mean yield of cells at end of **P4** as = $1.1x10^5$ cells
Given that 1.5392×10^5 cells = 3 population doublings, then 1.1×10^5 cells = **~3 pop. doublings.**

4 population doublings starting with 19,240 cells:

0.1924×10^5 cells x 2	$= 0.3848 \times 10^5$ cells =	1 population doubling
0.3848×10^5 cells x 2	$= 0.7696 \times 10^5$ cells =	2 population doublings
0.7696×10^5 cells x 2	$= 1.5392 \times 10^5$ cells =	3 population doublings
1.5392×10^5 cells x 2	$= \mathbf{3.0800 \times 10^5}$ **cells =**	**4 population doublings**

Bruder describes mean yield of cells at end of **P1** as = 2.7×10^5 cells
Given that 3.08×10^5 cells = 4 population doublings, then 2.7×10^5 cells = ~**4 pop. doublings.**

It is also noteworthy that Bruder comments, "This slowing of cell proliferation as a function of increasing passage number was independent of the feeding schedule used to maintain the cells" (*i.e.*, whether the cells were fed daily or twice weekly). *See*, Bruder, page 284, left column, second paragraph. This indicates that Haynesworth's "MSCs" acquire increasingly longer population doubling times independent of the cell culture conditions and, as such, their doubling rate is an inherent property of the cell population.

In sum, the cell populations isolated by Haynesworth differ from those of the present invention because:

1) Haynesworth's isolated cell population is CD44 negative, whereas cell populations of the present invention are CD44 positive;

2) Haynesworth's isolated cell population is obtained from a low density, platelet containing gradient fraction, whereas cells of the present invention are obtained from a high density mononuclear cell containing fraction;

3) Three specific media formulations are described as "*critical*" to isolation "*of only the mesenchymal stem cells*", whereas a different media formulation is used to isolate cells of the present invention;

4) Haynesworth's isolated cell population has reduced self-renewal capacity compared to cells of the present invention; and,

5) Haynesworth's isolated cell population acquires increasingly longer population doubling times with each passage in culture, whereas cell populations of the present invention maintain a doubling rate of less than about 30 hours even after 30 cell doublings.

Therefore, in view of the above described differences, the cell populations isolated by Haynesworth are not the same, nor are they essentially the same, as the cell populations of the presently pending claims.

C. THE CELLS DESCRIBED AND CLAIMED IN THE PRESENT PATENT APPLICATION ARE NOT THE SAME, NOR ARE THEY ESSENTIALLY THE SAME, AS THE CELL POPULATIONS DESCRIBED IN PITTENGER.

Pittenger describes an isolated population of cells which are CD106 positive. *See*, Pittenger, page 144, left column ("These expanded attached mesenchymal cells were uniformly positive for...CD106... No subpopulations of marrow derived mesenchymal cells could be discerned morphologically by microscopic observation or by fluorescence cytometry, size, and granularity criteria or with more than 50 available antibodies..."). Furthermore, the cells described in Pittenger are also CD62L positive. *See*, **Exhibit E;** Supplemental Web data at http://www.sciencemag.org/feature/data/983855.dtl (*cited in* Pittenger at page 143, right column, 1st paragraph).

Attached **Exhibit D** shows fluoresence activated cell-sorting (FACS) analysis of human bone marrow-derived cell populations isolated and cultured as described in the present patent application. The FACS data shown in **Exhibit D** was generated at Neuronyx Inc. (1 Great Valley Parkway, Suite 20, Malvern, PA), between March 2004 and March 2007. Each of these FACS experiments were carried out by someone under my direct supervision and control.

The rows of panels in **Exhibit D** are arranged showing FACS analysis performed on cell populations after about 18.8 population doublings (top row), after about 27.2 population doublings (second row), after about 35.2 population doublings (third row), after about 43.9 population doublings (fourth row), and after about 51.7 population doublings (bottom row). The columns for each of these panels show the results of FACS analyses using antibodies to detect CD62L (left column), CD106 (middle column), and CD13 (right column) versus an isotype negative control antibody. Notably, expression of each of these three markers remains constant from cell population doubling 18.8 (the earliest measurement) through cell population doubling 51.7 (the latest measurement). The cells used to generate the data in

Exhibit D were originally derived from a single human donor in February of 2004 using the same methods described in Application No. 09/960,244 (filed on Sept. 21, 2001).[2]

As demonstrated by **Exhibit D**, and in contrast to the CD106 and CD62L positive cell populations described in Pittenger, the isolated cell populations of the present invention are CD106 and CD62L negative. Therefore, the isolated cell populations of the present invention are not the same nor are they essentially the same as the cell populations described in Pittenger.

D. THE CELLS DESCRIBED AND CLAIMED IN THE PRESENT PATENT APPLICATION ARE NOT THE SAME, NOR ARE THEY ESSENTIALLY THE SAME, AS THE CELL POPULATIONS AS DESCRIBED IN LEE.

Lee describes a population of cells which are CD13 negative. *See*, Lee, page 1278, right column ("The fibroblast-like morphology of BM-derived cells...as well as their surface phenotype (Fig. 1B), as determined by flow cytometry, were consistent with those reported in the literature for MSCs [*citing* Pittenger, *Science* 1999; *284*:143-147]. These BM-derived *cells were negative for CD13...*") (emphasis added). Additionally, the cell populations described by Lee have longer doubling times than the cell populations of the presently claimed invention. In particular, Lee states "The doubling time of these BM-derived cells was found to be between 40 to 52 hours (data not shown)." *See*, Lee at page 1278, right column, 1st paragraph. In contrast to the cell populations described by Lee, the isolated cell populations of the present invention are CD13 positive, as demonstrated in **Exhibit D** (right column; discussed above). Therefore, the isolated cell populations of the present invention are not the same nor are they essentially the same as the cell populations described in Lee.

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[2] In particular, the cells were initially isolated according to the method described in Example 1 of the present application, wherein: a) a bone marrow aspirate was obtained from a healthy adult human volunteer; b) the aspirate was mixed with an ammonium chloride buffer to lyse the red blood cells; c) the mononuclear cells were pelleted and resuspended in complete media; d) the cells were seeded into tissue culture-treated containers at ~50,000 cells/cm^2; and, e) the cells were incubated at 37°C in an atmosphere consisting of ~5% carbon dioxide, ~5% oxygen, and ~90% nitrogen/air until adherent colony forming units were obtained. Thereafter, the cells were expanded and passaged according to the methods described in Examples 3-4 of the present application, wherein the cells were cultured under the same growth conditions described above while being re-seeded at a density of ~30 cells/cm^2 with each cell passage.

E. THE CELLS DESCRIBED AND CLAIMED IN THE PRESENT PATENT APPLICATION ARE NOT THE SAME, NOR ARE THEY ESSENTIALLY THE SAME, AS THE CELL POPULATIONS DESCRIBED IN WOODBURY.

The human "MSCs" described in Woodbury are the same as those described in Azizi *et al., Proc Natl Acad Sci USA.* 95:3908-3913 (1998) (hereinafter "Azizi"). This is evidenced by recitation in Woodbury that "hMSCs were isolated from a healthy adult donor and grown in vitro (Azizi et., 1998)." In this regard, Azizi notes that their isolated human "MSCs" are a heterogeneous population with at least two types of cells. In particular, Azizi notes "However, two distinct populations were seen, large flattened cells and relatively elongated or spindle shaped cells (Fig. 2a and b)." *See*, Azizi, page 3911, left column, last full paragraph. Azizi also characterizes their cell population as changing morphology in culture with continuing passaging in the same manner as discussed (*above*) in Bruder *et al.* (*i.e.*, same as for the "MSCs" of Haynesworth). *See*, Azizi, page 3911, left column, last full paragraph ("As noted previously (see ref. 24 [Bruder *et al.*]), the human MSCs became relatively homogeneous in appearance as the cells were passed."). Furthermore, the human "MSCs" of Azizi require cell culture supplementation with PDGF-AA for optimal growth. In contrast, as previously discussed in detail in my May 2007 Declaration, the cell populations of the present invention do not require growth factor supplementation.

Indeed, the "hMSCs" of Azizi required supplementation with PDGF-AA merely to grow sufficient quantities of the cell population to perform the experiments described therein. *See*, Azizi, page 3911, left column, first paragraph ("As indicated in Fig. 1, addition of PDGF-AA increased the growth rate of the cells. Therefore PDGF-AA was added to passages 2-5 to obtain adequate numbers of human MSCs for the experiments here."). Additionally, the "hMSCs" of Azizi have a population doubling time (PD) of about, at best, approximately 96 hours *with* PDGF-AA supplementation. *See*, Azizi, p. 3909, Fig. 1; showing cell number at Day 0 = $9x10^4$ cells, which approximately double, with PDGF-AA supplementation, to ~$18x10^4$ cells by Day 4 (*i.e.*, 96 hours later)).

Accordingly, given the requisite need for growth factor supplementation, the slow population doubling times, the presence of at least two distinct cell types, and the changing morphology of the cell population with continued passage, it is clear that the isolated cell populations of the present invention are not the same nor are they essentially the same as the cell populations described in Woodbury.

F. THE CELLS DESCRIBED AND CLAIMED IN THE PRESENT PATENT APPLICATION ARE NOT THE SAME, NOR ARE THEY ESSENTIALLY THE SAME, AS THE CELL POPULATIONS AS DESCRIBED IN JIANG.

The mouse cells described in Jiang are CD44 and MHC Class I *negative*. *See e.g.*, Jiang, page 41, right column ("The phenotype of cultured mMAPCs is CD34, CD44, CD45, c-Kit, and major histocompatibility complex (MHC) class I and II negative..."); *see also*, Fig. 1; *see also* Jiang, Y., *et al.*, "*Corrigendum*: Pluripotency of mesenchymal stem cells derived from adult marrow," *Nature 447*:879-880, Nature Publishing Company (June 2007). In contrast, as shown and discussed in my May 2007 Declaration the isolated cell populations of the present invention are CD44 and MHC Class I *positive*. Therefore, the isolated cell populations of the present invention are not the same nor are they essentially the same as the cell populations described in Jiang.

G. CONCLUSION

The phenotype of cells of the present invention differ substantially from the phenotype described for the cells in Haynesworth, Pittenger, Woodbury, Lee, and Jiang. In particular, the cells of the present invention differ in the consistent non-expression of CD62L and CD106 and in the consistently positive expression of CD44, HLA Class-1, and CD13. The cells of the present invention also differ in having consistent and significantly faster population doubling times of less than about 30 hours per population doubling (even after 30 population doublings). Thus, it is clear that the isolated cell populations of the present invention and the isolated cell populations described in the Haynesworth, Pittenger, Woodbury, Lee and Jiang references are different cell populations. In sum, the isolated cell populations of the present invention are *not* "MSCs," "MAPCs," or *mixed populations of*

"MSCs and MAPCs" such as described in the above referenced publications and according to the parameters by which these cells are generally understood and commonly recognized by those practicing in the field of stem cell biology.

H. AFFIRMATION

I further declare that the above statements made of my own knowledge are true and the above statements based on information and belief obtained from the references and documents discussed are believed to be true. Additionally, I declare that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Title 18 United States Code Section 1001, and that willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Respectfully submitted,

Gene C. Kopen, Ph.D.

Date: 03-March-08

Exhibit A

CURRICULUM VITAE

Name: Gene C. Kopen, Ph.D.

Address: 609 Argyle Rd.
Wynnewood, PA 19096
Home: 610-896-9001

Personal: Married, 3 children

Work Address: Neuronyx, Inc.
1 Great Valley Parkway, Suite 20
Malvern, PA 19355
Phone (610) 240-4157
Email: gkopen@neuronyx.com

RESEARCH AND RELATED PROFESSIONAL EXPERIENCE

I joined Neuronyx in June, 2000 as the 6th employee and was made responsible for recruiting and training R&D and animal facility staff, developing and implementing methodologies for large scale expansion of human stem cells, developing a framework for product development and testing, initiating collaborations with thought leaders in the field of neurodegeneration, and establishing an in-house pre-clinical program in spinal cord injury. By 2003 the company had approximately 51 employees, a GLP-compliant animal facility, cell manufacturing capability, and additional departments of Quality Control, Quality Assurance, and Clinical and Regulatory.

11/2007-Present **Vice President, Technology Development and IP management, Neuronyx, Inc.**

6/2007-10/2007 **Sr. Director, Technology Development, Neuronyx, Inc.**

2003-5/2007 **Sr. Scientist, Neuronyx, Inc.**

Responsibilities Included:

- Developing new clinical program opportunities leveraging platform technology
- Developing strategic R&D objectives supporting/driving operating plans

- Managing milestone driven projects leading to value-adding product-development and process-development opportunities
- Managing Intellectual property portfolio
- Developing new technologies for next generation of biopharmaceuticals
- Directing core projects aimed at Cell Product characterization
- Managing IND-enabling pre-clinical research conducted through key academic collaborations and contract research organizations

Accomplishments:

- Part of cardiovascular IND team leading to compilation, submission and clearance of Phase I clinical Trial in Sub-Acute Myocardial Infarction (NCT00361855)
- Part of Manufacturing team leading to GMP processes for Manufacture of clinical-grade human ABM-SC, NX-CP015
- Portfolio of pharmacokinetic and toxicology studies supporting Stroke clinical development. Efficacy studies conducted through sponsored research lead to Grant from Department of Health and Human Services, State of Illinois.
- Development of bioassay systems and testing used to establish technical specifications for Manufacturing and Quality Control

2000-2003 **Scientist, Neuronyx, Inc.**

Responsibilities included:

- Development of technologies for isolation and *ex vivo* expansion of various human stem cells and somatic cells
- Implemented animal model of spinal cord injury (SCI) to study therapeutic potential of cell prototypes; trained and managed LAF staff, developed protocols for surgical & LAF procedures, GLP pre-clinical studies, etc.
- Managed many projects aimed at cell differentiation, characterization, and manufacture, as well as, pre-clinical development of cell prototypes
- Recruited and trained key positions for R&D and early phase-Manufacturing
- Managed IND-enabling pre-clinical research conducted through key academic collaborations and contract labs

Accomplishments:

- Developed *ex vivo* expansion technologies for human stem cells; this early work was quickly enabled for scale-up by other members of the team, leading to our current GMP manufacturing capacity of approximately 5 x 10^9 clinical doses of human ABM-SC from a single donor

- Assembled a pre-clinical SCI team in tandem with an academic collaboration that lead to 11+ pre-clinical studies supporting path to IND

1999-2000 Post-Doctoral Fellow, Center for Gene Therapy, MCP Hahnemann University, Philadelphia, PA.

Selected Scientific Projects (much of this work was initiated in parallel with my dissertation research):

- Development of new methods for isolation and *ex vivo* expansion of bone marrow stromal cells (MSC)
- Characterization of murine and human-derived MSC by flow cytometry, *in vitro* differentiation, *in vivo* pharmacology, as ell as various molecular techniques
- Identification of therapeutic potential of MSC in animal model of storage disease
- Development of *in vitro* differentiation assays aimed at mesenchymal-to-epithelial differentiation of MSC

1995-1999 Graduate Student. Doctoral Program Molecular Pathobiology, MCP Hahnemann University, Philadelphia, PA.

Mentor: Darwin Prockop M.D, Ph.D. Center for Gene Therapy

Thesis: Murine Bone Marrow Stromal Cells: Characterization, Isolation, and Differentiation

Other: Vice President, Student Government, Graduate Admissions committee member

1993-1995 Clinical Laboratory Manager, Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA

Responsibilities included:

- Supervision of all facets of diagnostic Immunohistochemistry (IHC) and assay development for 10 + area hospitals and research institutions
- Conducted Image analyses of breast cancers for ER/PR
- DNA ploidy by Flow Cytometry
- Conducted diagnostic Immunofluorescence on renal and skin bx
- Stat cryosectioning and histology on surgically resected bx
- Training cytology students and local researchers on methods of IHC

- Developed and optimized techniques for diagnostic detection of various tissue antigens
- Patient billing and lab QC
- Other duties included examination, processing, and dictation of gross exam on surgical specimens. Autopsy duties, when needed, included organ removal and processing for histopathology

1992-1995 Research Associate, (part-time) **Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA.** Laboratory of James England, MD., Ph.D.

Lab focus was study of oncogenic events responsible for mesothelioma formation and *V-SRC* mediated tumor formation and metastasis.

1992 Student Volunteer, Labor and Delivery, Ob/Gyn, Hospital of the University of Pennsylvania, Phila., PA. (Part-time, Summer)

Volunteer training program. Program objective to give pre-doc students clinical exposure.

1990-1992 Research Associate, Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA. (Full-time) Laboratory of James England, MD., Ph.D.

Lab focus was study of immunologic mechanisms controlling *V-SRC* induced tumor cell growth and metastasis. This work was conducting in collaboration with Michael Halpern, Ph.D, of the Wistar Institute, Philadelphia, PA.

1990 Psychiatric Technician, Eastern Pennsylvania Psychiatric Institute (Full-time, Summer)

Principle responsibilities included, conducting patient history & physical assessment for new admissions. Patient support & monitoring, maintaining patient compliance to therapeutic regiments.

1988 Sales & Marketing, Eastern Telephone Systems, Fort Washington, PA. (Full-Time)

Sales and marketing of commercial services, client services.

1987 Internship, Kidder, Peabody, & Co, Tampa, Fl. Brokerage and Client Services.

Exhibit A

EDUCATION AND TRAINING

Univ. of South Florida, Tampa, FL	**B.A. Social & Behavioral Sciences, 1987**
Univ. of Pennsylvania, Phila., PA	**Post-Baccalaureate Program, Pre-Health, 1991-1994**
Drexel College of Medicine, Phila., PA	**Ph.D. Molecular Pathobiology, 1999**

PUBLICATIONS

England, J.M., Panella, M.J., **Kopen, G.C.**, Wisner, T.W., and Halpern, M.S. (1994) Tumor cells induced by the v-src oncogene are heterogeneous for expression of markers of mesenchyme differentiation. Virchows Archiv. 424 (1): 83-8.

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Appelt, D.M., **Kopen, G.C.**, Boyne, L.J., and Balin, B.J. (1996) Localization of transglutaminase in hippocampal neurons: implications for Alzheimer's disease. J. of Histo.& Cytochem. Dec; 44 (12): 1421-7.

Taylor, R.L., England, J.M., **Kopen,G.C.**, Christou, A.A., and Halpern, M.S. (1996) Sequence variation in the src gene product affects metastasis formation: the central, but not exclusive, role of the tumor immune response. International J. of Cancer. Oct. 9; 68 (2): 228-31.

Halpern, M.S., England, J.M., **Kopen, G.C.**, Christou, A.A., and Taylor, R.L. (1996). Endogenous c-src as a determinant of the tumorigenicity of src oncogenes. Proceedings of the National Academy of Sciences of the United States. 93 (2): 824-7.

Li,S.W., Arita, M., **Kopen.G.C.**, Phinney, D.G., and Prockop, D.J. (1998) A 1,064 bp fragment from the promoter region of the col11a2 gene drives LacZ expression not only in cartilage but also in osteoblasts adjacent to regions undergoing both endochondrial and intermembranous ossification in mouse embyos. Matrix Biology. July; 17(3): 213-221.

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Kopen, G.C., Prockop, D.J., and Phinney, D.G. (1999) Enhanced in situ detection of β-glucuronidase activity in murine tissue. Journal of Histochemistry and Cytochemistry. 47(7): 965-968.

Kopen, G.C., Prockop, D.J., and Phinney, D.G. (1999) Marrow stromal cells migrate throughout forebrain and cerebellum and they differentiate into astocytes following injection into neonatal mouse brain. Proc Nat'l Acad Sci USA. 96:10711-10716.

Prockop, D.J., Azizi, S.A., Colter, D., DiGirolamo, C., **Kopen, G.C.**, and Phinney, D.G. 2000. Potential use of stem cells for bone marrow to repair the extracellular matrix and the central nervous system. Biochem. Society Transactions. 28(4): 341-5.

Prockop DJ, Azizi SA, Phinney DG, Kopen GC, Schwarz, EJ. 2000. Potential use of marrow stromal cells as therapeutic vectors for diseases of the central nervous system. Prog. Brain Res. 128: 293-7.

Li, S., Arita, M., Fertala, A., Bao, Y., **Kopen, G.C.**, Langsjo, T., Helminen, H.J., and Prockop, D.J. 2001. Transgenic mice with inactive alleles for procollagen N-proteinase (ADAMTS-2) develop fragile skin and male sterility. Biochem. J. Apr 15:355 (pt2): 271-8.

Arita M, Li SW, **Kopen G**, Adachi E, Jimenez SA, Fertala A. 2002. Skeletal abnormalities and ultrastructural changes of cartilage in transgenic mice expressing a collagen II gene (COL2A1) with a Cys for Arg-alpha1-519 substitution. Osteoarthritis Cartilage. Oct: 10(10): 808-15.

Tremain N, Korkko J, Ibberson D, **Kopen GC**, DiGirolamo C, Phinney DG. 2001. MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. Stem Cells. 19(5): 408-18.

Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, **Kopen GC**, Phinney DG. 2003. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. J Cell Biochem. Aug 15; 89(6):1235-49.

Himes BT, Neuhuber B, Coleman C, Kushner R, Swanger SA, **Kopen GC**, Wagner J, Shumsky JS, Fischer I. 2006 Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. Neurorehabil. Neural Repair. Jun; 20(2): 278-96.

Andrews EM, Tsai SY, Johnson, SC, Farrer JR, Wagner JP, **Kopen GC**, Kartje, GL. 2008. Human adult bone marrow-derived somatic cell therapy results in functional recovery and axonal plasticity following stroke in the rat. Experimental Neurology. *In Press*

EXHIBIT B

SIGMA-ALDRICH®

LEUKOCYTE SEPARATION

(Procedure No. 1119)

INTENDED USE

A method using HISTOPAQUE®-1119 and HISTOPAQUE®-1077 for separating mononuclear cells and granulocytes. HISTOPAQUE®-1119 reagents are for "In Vitro Diagnostic Use".

In 1968, Boyum[1] described gradient density centrifugation methods for isolation of mononuclear cells from circulating blood and bone marrow. Solutions used for this purpose consist of a polysucrose and a radiopaque medium. HISTOPAQUE®-1077 is such a solution adjusted to a density of 1.077. When blood is layered onto HISTOPAQUE®-1077 and subjected to centrifugal forces, mononuclear cells are held at plasma HISTOPAQUE® interphase while erythrocytes and granulocytes gravitate to the bottom. It would appear feasible to devise a system whereby cells of the myeloid series could also be harvested employing a one-step procedure. HISTOPAQUE®-1119 was developed to achieve this purpose and is based on observations by English and Andersen.[2]

According to the Sigma-Aldrich procedure, a double gradient is formed by layering an equal volume of HISTOPAQUE®-1077 over HISTOPAQUE®-1119. Whole blood is carefully layered onto the upper HISTOPAQUE®-1077 medium. The tubes are then centrifuged at 700 x g for 30 minutes. Cells of the granulocytic series are found at the 1077/1119 interphase whereas lymphocytes, other mononuclear cells and platelets are found at the plasma/1077 interphase.

REAGENT

HISTOPAQUE®-1119, Catalog No. 1119-1
Polysucrose, 6.0 g/dl and sodium diatrizoate, 16.7 g/dl. Aseptically filtered.

STORAGE AND STABILITY:
Store HISTOPAQUE®-1119 in refrigerator (2–8°C). Protect from light. Reagent label bears expiration date.

DETERIORATION:
A cloudy appearance indicates deterioration of the product.

PREPARATION:
HISTOPAQUE®-1119 is ready for use. Warm to 18–26°C before use.

PRECAUTIONS:
Normal precautions exercised in handling laboratory reagents should be followed. Upon contact with human source substances, treat all reagents and equipment as potentially biohazardous. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

US Risks and Safety Statements
HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing. Target organ: Blood.

EU Risks and Safety Statements
HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Do not breathe vapor. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

PROCEDURE

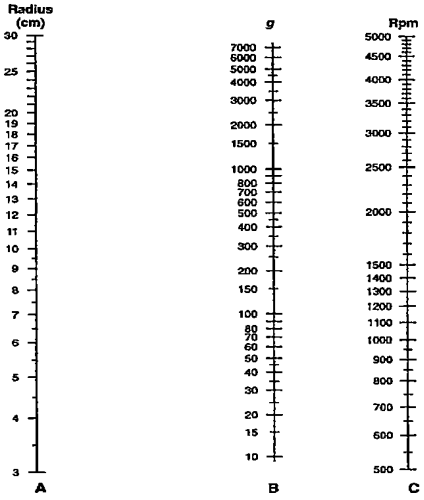
SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Collect 6 ml venous blood in preservative-free heparin or EDTA.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
HISTOPAQUE®-1077, Catalog No. 1077-1, Polysucrose, 5.7 g/dl, and sodium diatrizoate, 9.0 g/dl. Aseptically filtered.
Centrifuge (Swinging Bucket Rotor) capable of generating 700 x g
Centrifuge tubes, 15 ml plastic, conical

NOTES:
1. 50 ml centrifuge tubes may be used. Alter stated procedure by using 12 ml of HISTOPAQUE®-1077, 12 ml of HISTOPAQUE®-1119, and 24 ml of whole or diluted blood.
2. On occasion it may be necessary to dilute blood 1:2 or 1:4, depending upon absolute cell numbers. The possibility of overloading the gradient exists.
3. Avoid use of powdered gloves. Glove powder will activate monocytes and cause lower yields.
4. Avoid use of high binding plastics such as polystyrene. Polystyrene may bind cells to the centrifuge tube walls.
5. Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur and result in poor cell recovery.
6. Underlaying the HISTOPAQUE®-1119 will also produce an acceptable gradient.
7. Other anti-coagulants may be used; however the choice of anti-coagulant may affect cell recovery. As blood ages the cell recoveries will drop.
8. The procedure section of this insert employs use of isotonic phosphate buffered saline as a diluent and washing fluid. Other reagents such as cell medium RPMI 1640 supplemented with fetal bovine serum may be used.
9. The procedure described is for isolation of cells from 6 ml of whole blood. Volumes may be increased or decreased as necessary.
10. The use of a "normal" patient is recommended as a control for each run.

NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGAL FORCES:

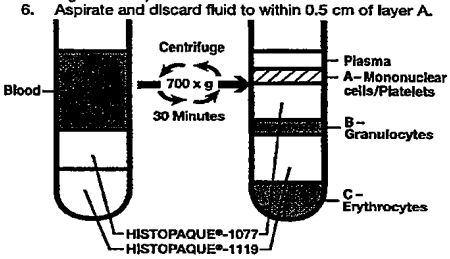


A nomogram is used to derive the rpm setting for your centrifuge.

How to establish the rpm required to obtain 700 x g for Procedure No. 1119.
1. Measure the radius (cm) from the center of the centrifuge spindle to the end of the test tube carrier. Mark this value on scale A.
2. Mark the relative centrifugal force (e.g., 700) on scale B.
3. With a ruler, draw a straight line between points on columns A and B, extending it to intersect column C. The reading on column C is the rpm setting for the centrifuge.

PROCEDURE:
1. Add 3 ml HISTOPAQUE®-1119, to a 15 ml conical centrifuge tube.
2. Carefully layer 3 ml of HISTOPAQUE®-1077, onto the HISTOPAQUE®-1119.
3. Carefully layer 6 ml of whole blood onto the upper gradient of the tube from Step 2.
4. Centrifuge at 700 x g for 30 minutes at room temperature (18–26°C). Centrifugation at lower temperatures, such as 4°C, may result in cell clumping and poor recovery.

NOTE: The rpm required to generate 700 x g can be calculated using the nomogram in this insert.
5. Carefully remove centrifuge tubes. Two distinct opaque layers should be observed (layers A and B in Figure below).
6. Aspirate and discard fluid to within 0.5 cm of layer A.



Transfer cells from this layer to a tube marked "mononuclear".
7. Aspirate and discard remaining fluid to within 0.5 cm of layer B. Transfer cells from this layer to a tube labeled "granulocytes".
8. Wash the cells by addition of 10 ml isotonic phosphate buffered saline to the tubes. Centrifuge 10 minutes at 200 x g. Remove the supernatant and discard.
9. Resuspend the cells by gentle aspiration with a Pasteur pipet.
10. Repeat Steps 8 and 9 two times.
11. Resuspend cells in an appropriate volume of isotonic phosphate buffered saline.

At this point a variety of assays can be performed. The procedures are chosen according to individual discretion.

PERFORMANCE CHARACTERISTICS

Erythrocytes should pellet to the bottom of the centrifuge tube. Granulocytes should band at the interface between the HISTOPAQUE®-1119 and the HISTOPAQUE®-1077. Mononuclear cells should band at the interface between the HISTOPAQUE®-1077 and the plasma

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Boyum A: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21: suppl 97:77, 1968
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Procedure No. 1119
Previous Revision: 2003-04
Revised: 2003-09

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Exhibit C

Component	09/960,244 Medium	Haynesworth Media		
	Alpha MEM (mg/L)	DMEM (mg/L)	BGJB Medium (mg/L)	Ham F12 (mg/L)
$CaCl_2$ $2H_20$				44
$CaCl_2$ (anhydrous)	200.0	200.0		
$CuSO_4$ $5H_20$				0.00249
$FeSO_4$ $7H_20$				0.834
KCl	400.0	400.0	400.00	223.6
MgCl2 6H20				122
$MgSO_4$ (anhydrous)	97.7	97.7	200.00	
KH_2PO4			160.00	
NaCl	6800.0	6400.0	5300.00	7599
Calcium Lactate			550.00	
$NaH_2PO_4 \cdot H_2O$	140.0	125.0	90.00	268
$ZnSO_4$ $7H_20$				0.863
L-Alanine	25.0		250.00	8.9
L-Arginine HCl	127.0	84.0	175.00	211
L-Asparagine H_2O	50.0			15.01
L-Aspartic Acid	30.0		150.00	13.3
L-Cysteine HCl·H_2O	100.0		101.00	35.12
L-Cystine 2HCl	31.3	662.6		
L-Glutamic Acid	75.0			14.7
L-Glutamine	292.0	584.0	200.00	146
Glycine	50.0	30.0	800.00	7.5
L-Histidine HCl·H_2O	41.9	42.0	150.00	20.96
L-Isoleucine	52.5	105.0	30.00	3.94
L-Leucine	52.4	105.0	50.00	13.1
L-Lysine HCl	72.5	146.0	240.00	13.5
L-Methionine	15.0	30.0	50.00	4.48
L-Phenylalanine	32.0	66.0	50.00	4.96
L-Proline	40.0		400.00	34.5
L-Serine	25.0	42.0	200.00	10.5
L-Threonine	48.0	95.0	75.00	11.9
L-Tryptophan	10.0	16.0	40.00	2.04
L-Tryosine 2Na·$2H_2O$	51.9	103.8	40.00	5.4
DL-Valine			65.00	
L-Valine	46.0	94.0		11.7
L-Ascorbic Acid	50.0		50.00	
d-Biotin	0.1		0.20	
D-Ca Pantothenate	1.0	4.0		
Choline Chloride	1.0	4.0	50.00	
Folic Acid	1.0	4.0	0.20	
Myo-Inositol	2.0	7.2	0.20	
Nicotinamide			20.00	
Niacinamide	1.0	4.0		
para-aminobenzoic acid			2.00	
Pyridoxal HCl	1.0	4.0	0.20	

Riboflavin	0.1	0.4	0.20	
Thiamine HCl	1.0	4.0	4.00	
Vitamin B-12	1.4		0.04	
D-Glucose	1000.0	1000.0	101000.00	1802
Lipioc Acid	0.2			0.21
Phenol Red (Sodium)	11.0	15.0	20.00	1.2
Sodium Pyruvate	110.0	110.0		110
$NaHCO_3$	2200.0	3700.0		1176
Sodium Acetate			50.00	
α-tocopherol phosphate (disodium salt)			1.00	
Thymidine				0.73
Putrescine 2HCl				0.161
Linoleic Acid				0.084
Hypoxanthine				4.1

Exhibit D

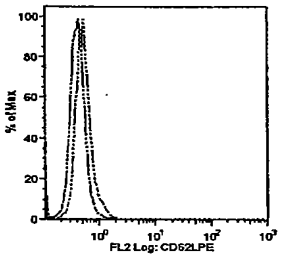
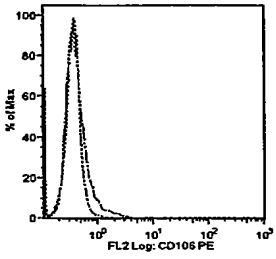
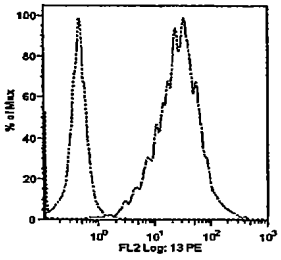
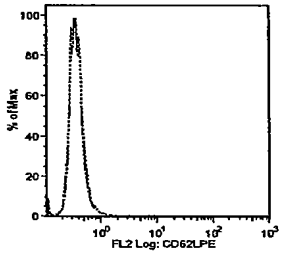
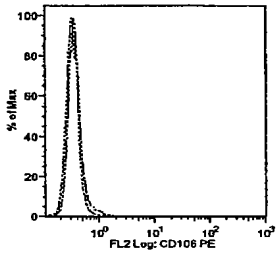
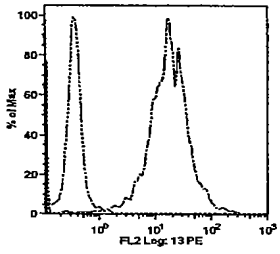
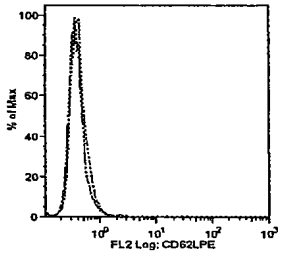
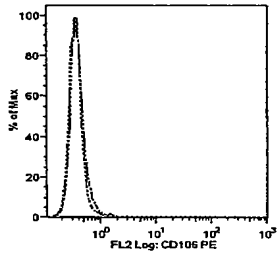
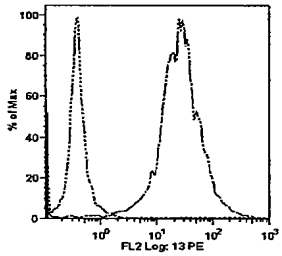
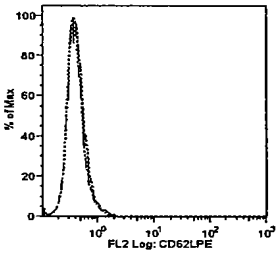
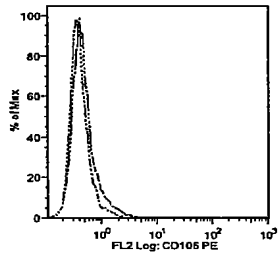
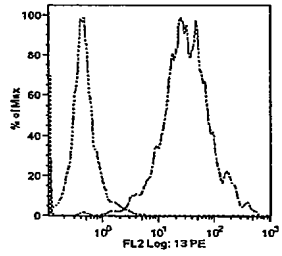
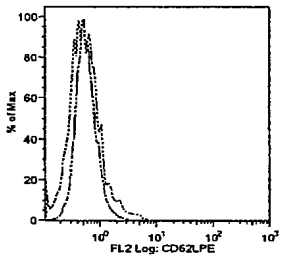
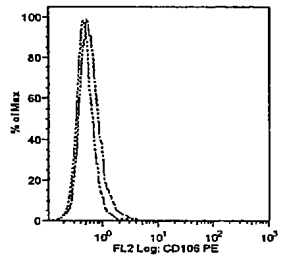
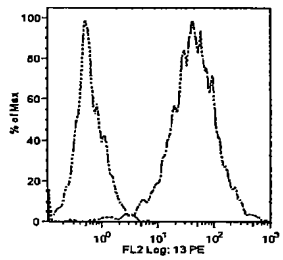
	anti-**CD62L** (blue) vs neg. control ab. (red)	anti-**CD106** (blue) vs neg. control ab. (red)	anti-**CD13** (blue) vs neg. control ab. (red)
18.8 PD			
27.2 PD			
35.2 PD			
43.9 PD			
51.7 PD			

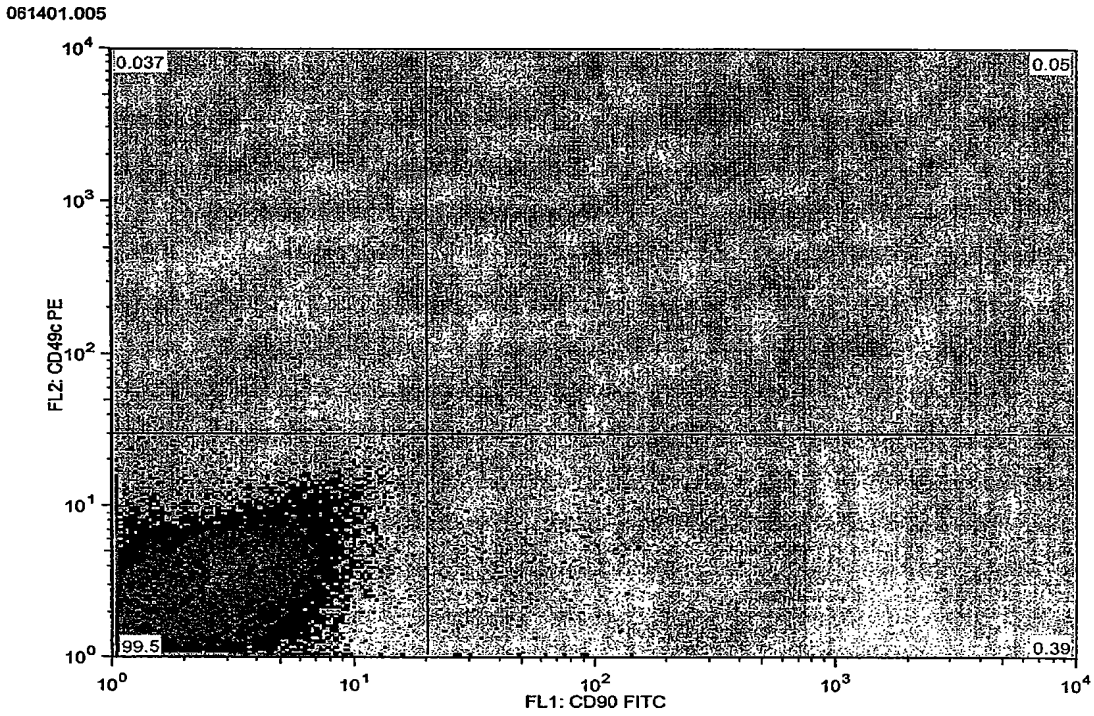
Exhibit E

CD49c/CD90 FACS Analysis of Cells Isolated Via Ammonium Chloride Lysis or HISTOPAQUE® 1.119 Gradient Separation Procedures

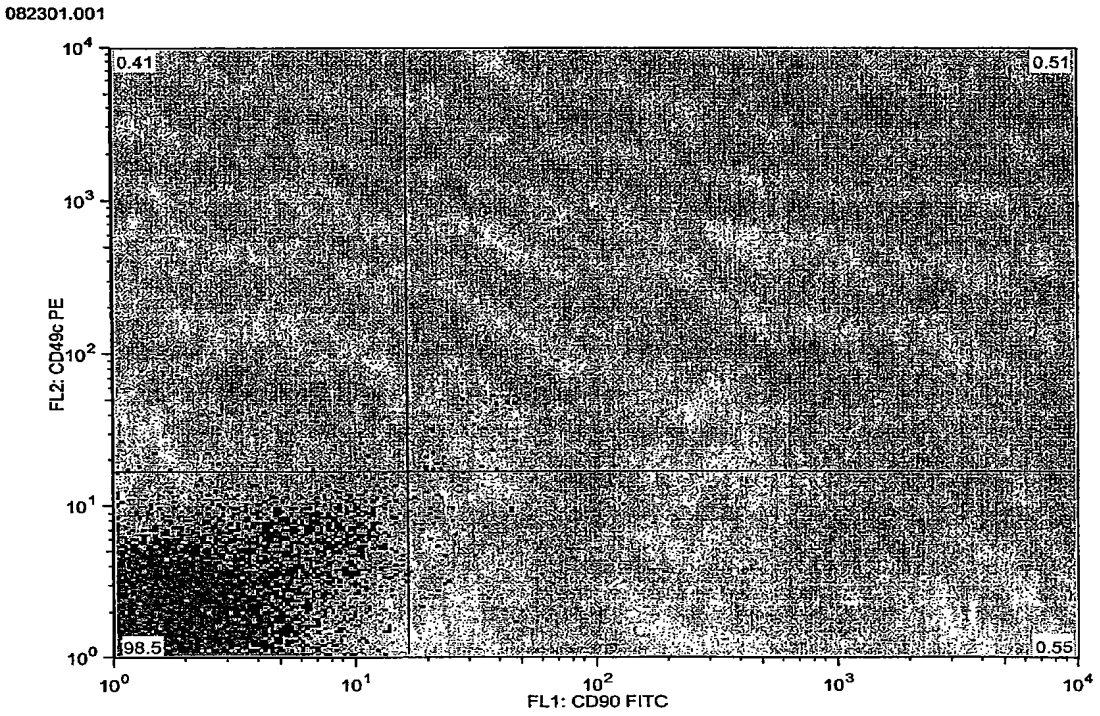
FACS analysis was performed on aspirates of human adult bone marrow-derived cells prepared as described in Example 1 (red blood cell/ammonium chloride lysis procedure) and Example 2 (density gradient separation procedure) of U.S. Patent Application 09/960,244.

Resuspended cells (approximately 10^6) were aliquoted into12x75mm Flow Cytometry tubes and repelleted at 500xg for 5 minutes. The HBSS was removed and 25 microliters of the following antibodies (all obtained from Becton Dickenson), alone or in combination, were placed into each tube: mouse IgG1k-FITC or -PE (clone MOPC-21) CD49c-PE (cl. C3II.1), CD90-FITC (cl. 5E10). Tubes were gently vortexed and incubated for 30 minutes at 4°C. Cells were then washed in HBSS/1% bovine serum albumin, centrifuged (30 min, 4°C) and the resulting cellular pellet fixed by the addition of 250 microliters of 2% paraformaldhyde/HBSS. Flow cytometric analysis was performed employing a Becton-Dickenson FACSVantage SE cytometer and analyzed using CellQuest® software. Depicted results represent data collected from 2,500- 10,000 events per panel.

After compensation for non-specific antibody staining using mouse IgG1 isotype controls, cellular expression of CD49c and CD90 in the bone marrow cells was assessed. As seen in Panel A, after red blood cell/ammonium chloride lysis only, approximately 0.05% of recovered cells co-express CD49c and CD90 (upper right quadrant). As seen in Panel B, after density gradient separation using HISTOPAQUE® 1.119, the fraction of cells co-expressing CD49c and CD90 was approximately 10 fold higher at 0.51% than the concentration obtained via the red blood cell/ammonium chloride lysis procedure.



Panel A



Panel B